

Figure 10a shows a plasmid DNA separation kit based on compaction agent precipitation technology. In the box a total of 8 solutions should be include two of which are optional. Solutions will include the 3 common alkaline lysis solutions, a low ionic strength resuspension buffer, a compaction agent precipitation solution, and a stripping solution, and optionally a 70% ethanol wash solution and a final resuspension solution.

Description of each bottle are as follows:

- A. Alkaline lysis solution I (e.g. 25 mM Tris and 10 mM EDTA at pH 8.0)
- B. Alkaline lysis solution II (e.g. 1% Sodium Dodecyl Sulfate (SDS) 0.2 N NaOH)
- C. Alkaline lysis solution III (e.g. 3 M KAc at pH 5.5)
- D. Reuspension solution (e.g. 10 mM Tris at pH 8.0)
- E. Compaction agent precipitation solution (e.g. 2 mM Spermidine 3HCl and 10 mM Tris at pH 8.0
- F. Compaction agent stripping solution (e.g. 50% EtOH, 300 mM NaCl, 12.5 mM EDTA\*)
- G. 70% EtOH wash\*
- H. Final Resuspension solution (e.g. High purity TE which is 10 mM Tris and 1 mM EDTA at pH 8.0).

\*User may have to add enough EtOH to bring the solution to the proper percentage of EtOH.

\*Optional kit components that could be provided by end user.

Also, solutions D and E can be combined to form a resuspension/compaction agent precipitation solution.

Figure 10b shows an RNA isolation kit based on a hexammine cobalt precipitation process.

A. Lysis/DNA precipitation solution (e.g. 50% BPER with 2.5 mM Spermidine or 1% Brij 58 with 2.5 mM spermidine in 10 mM bis tris propane at pH 7). This may also be extended for use with plant cells and other eukaryotic cells with the possibilities of homogenization.



- other lysis solutions, and breaking the lysis from the spermidine DNA removal. Thus, there would be a lysis solution and a separate DNA precipitation solution.
- B. Hexammine cobalt precipitation solution (e.g. 7 mM hexammine cobalt for a total RNA precipitation or 4 mM hexammine cobalt in 10 mM bis tris propane at pH 6.9 for a high molecular weight precipitation).
- C. Optional: a second hexammine cobalt precipitation solution to bring down low molecular weight RNA not precipitated when solution B was used (e.g. 20 mM hexammine cobalt in 10 mM bis tris propane at pH 6.9).
- D. Stripping solution (e.g. 50% isopropyl alcohol\* with 3 M Urea, 300 mM NaCl, 25 mM EDTA).
- E. 70% EtOH wash\*,#
- F. Final Resuspension solution (e.g. High purity TE which is 10 mM Tris and 1 mM EDTA at pH 8.0).#

"User may have to add enough alcohol to bring the solution to the proper percentage of alcohol.

"Optional kit components that could be provided by end user.

Clean Copy of Insert to be substituted for page 32, line 19 to page 35, line 13:

## **Brief Description of the Drawings**



Figure 1 is a schematic diagram of preferred structures of common compaction agents

**Figure 2** shows schematically the precipitation by spermidine of 40  $\mu$ g/mL pBGS19luxwt or Baker's yeast RNA in 10 mM Tris buffer at pH 8.0 with and without 600 mM NaCl. (Error bars are +/- one standard deviation.)

Figure 3. Depicts a 1% agarose gel tracing the large-scale purification of pBGS19luxwt plasmid DNA. Lane 1 is a supercoiled plasmid ladder from Gibco; Lane 2 is the preparation after Celite filtration, isopropanol precipitation, and resuspension; Lane 3 is the supernatant after LiCl precipitation; Lane 4 is the supernatant of the compaction precipitation by 2.9 mM spermidine HCl; Lane 5 is the resuspended pellet of the compaction precipitation after stripping of spermidine by 300 mM NaCl, 10 mM MgCl2, and 25 mM EDTA in 50% isopropanol; Lane 6 is a 10X loading of the material in Lane 5 (The traces of genomic DNA in these lanes can be removed by further optimization of the initial lysis and precipitation steps); Lane 7 is after a Q Sepharose anion-exchange column (See Figure 4, bottom, Peak 5); Lane 8 is a 10X loading of Lane 7 and Lane 9 is pBGSi9LUXWT plasma DNA separated using the miniprep procedure.[the same as Lane 1].

Cont

Figure 4. Shows the chromatograms from a Pharmacia FPLC System using a HP Q Sepharose anion-exchange separation of pBGS19luxwt of an alkaline lysate after isopropanol and LiCl precipitation and optional compaction precipitation. Top: NaCl gradient; Middle: with no previous compaction precipitation step; Bottom: identical separation after a compaction precipitation step (1 volume of 2.9 mM spermidine in 10 mM Tris HCl at pH 8.0; see example 1). A Spectrum chromatography column (2.5 cm x 60 cm) packed with 150 mL Q Sepharose high performance media and equilibrated in 10 column volumes of TE with 570 mM NaCl is used. Loading and elution are performed at a linear velocity of 90 cm/hr.

**Figure 5** shows schematically <u>a summary of selective precipitation-based noncolumn DNA purification</u> process steps for separation of DNA as disclosed in Example 1.

**Figure 6.** shows a 3% Biogel (from Bio101 Inc.) electrophoretic analysis of *V. proteolyticus* RNA purified by Example 9. Lane 1 is the Ambion RNA Century Plus Size Markers; Lane 2 is the lysate after BPER addition, spermidine addition, and centrifugation; Lane 3 is the supernatant of the 4 mM hexammine cobalt precipitation; and Lane 4 is the RNA pelleted in the hexammine cobalt precipitation but before any column separation.

**Figure 7**. shows a FPLC chromatogram of *V. proteolyticus* RNA on a 25 mL high performance Q Sepharose anion exchange column (Pharmacia). The gradient ran over 12 column volumes from 0.30 M NaCl to 0.57 M

Cont

NaCl in a column buffer of 20 mM bis-tris propane and 20 mM EDTA at pH 6.9. (see Example 9)

**Figure 8** shows a FPLC chromatogram of pCP3X3 aRNA-containing *E. coli* strain JM109 on a 25 mL high performance Q Sepharose anion-exchange column (Pharmacia). The gradient is run over 12 column volumes from .37 M NaCl to .57 M NaCl in a column buffer of 20 mM bis-tris propane and 20 mM EDTA at pH 6.9. (see Example 10)

**Figure 9** shows a FPLC chromatogram of selective precipitation purified  $\beta$  ribozyme on a 25 mL high performance Q Sepharose anion exchange column (Pharmacia). The gradient is run over 12 column volumes from .37 M NaCl to .7 M NaCl in a column buffer of 10 mM bis-tris propane and 2 mM EDTA at pH 6.9. (see Example 11)

**Figure 10** shows schematically a kit for convenient practice of the invention. Figure 10a shows a plasmid DNA separation kit and Figure 10b shows an RNA isolation kit, both further described under "Description of Exemplary Kits".